

Claims:

1. A method of identifying a G protein coupled receptor signaling inhibitor, which comprises:
 - (a) providing a peptide library based on a native G protein coupled receptor binding peptide;
 - 5 (b) screening said peptide library for high affinity binding to said G protein coupled receptor;
 - (c) selecting a member of said peptide library having binding to said G protein coupled receptor of higher affinity than that of the native peptide;
 - 10 (d) providing a library of candidate compounds to screen for binding to said G protein coupled receptor;
 - (e) screening said library of candidate compounds for high affinity binding to said G protein coupled receptor in competition with a member of said peptide library selected in step (c); and
 - 15 (f) identifying a member of said library of candidate compounds having binding to said G protein coupled receptor of equal or higher affinity than that of the peptide selected in step (c).
2. A method of claim 1, wherein said screening of step (b) or step (e) is performed by testing for binding to an intact G protein coupled receptor.
3. A method of claim 1, wherein said screening of step (b) or step (e) is performed by testing for binding to at least an intracellular fragment of a G protein coupled receptor.

4. A method of claim 1, wherein said G protein coupled receptor binding peptide of step (a) is a G protein subunit or fragment thereof.

5. A method of claim 4, wherein said G protein subunit fragment is from about 7 to about 70 amino acids long.

6. A method of claim 4, wherein said G protein subunit fragment is from about 7 to about 55 amino acids long.

7. A method of claim 4, wherein said G protein subunit fragment is about 8 to about 50 amino acids long.

8. A method of claim 4, wherein said G protein subunit fragment is about 9 to about 23 amino acids long.

9. A method of claim 4, wherein said G protein subunit fragment is about 11 amino acids long.

10. A method of claim 4, wherein said G protein subunit is a G α subunit.

11. A method of claim 4, wherein said G protein coupled receptor binding peptide is a G α subunit carboxyl terminal peptide.

12. A method of claim 4, wherein said G protein subunit is a G $\beta\gamma$ dimer.

13. A method of claim 1, wherein said screening of step (b) comprises at least two sequential binding assays.

14. A method of claim 13, wherein at least one of said sequential binding assays is a competitive binding assay.

15. A method of claim 1, wherein said screening of step (b) is a competitive binding assay.

16. A method of claim 14, wherein said competitive binding assay is characterized by co-incubation of members of said peptide library with said G protein coupled receptor binding peptide.

17. A method of claim 15, wherein said competitive binding assay is characterized by co-incubation of members of said peptide library with said G protein coupled receptor binding peptide.

18. A method of claim 15, wherein said peptide library members are capable of providing a detectable signal.

19. A method of claim 1, wherein said candidate compounds of step (e) are capable of providing a detectable signal.

20. A method of claim 1, wherein said screening is an enzyme-linked immunosorbant assay.

21. A method of claim 1, wherein binding to said G protein coupled receptor is determined by measuring a signal

generated from interaction of an activating ligand with said G protein coupled receptor.

22. A method of claim 21, wherein activation of said G protein coupled receptor is determined.

23. A method of claim 21, wherein inhibition of said G protein coupled receptor is determined.

24. A method of claim 1, wherein said peptide library is a combinatorial peptide library.

25. A method of claim 24, wherein said combinatorial peptide library is a protein-peptide fusion protein library.

26. A method of claim 25, wherein said protein-peptide fusion protein library is a maltose binding protein-peptide fusion protein library.

27. A method of claim 1, wherein said peptide library is a peptide display library.

28. A method of claim 1, wherein said library of candidate compounds of step (d) is a focused library of candidate compounds based on the structure of a compound selected in step (c).

29. A method of claim 20, wherein said enzyme-linked immunosorbant assay comprises the steps of:

(a) immobilizing said G protein coupled receptor onto a solid support;

C
O
D
E
B
R
O
U
G
H
T
S

5 (b) providing a protein-peptide fusion protein display library;

 (c) incubating members of said protein-peptide fusion protein display library with said immobilized G protein coupled receptor in the presence of said G protein coupled receptor binding peptide under conditions such that members of protein-peptide fusion protein display library having a binding affinity for said G protein coupled receptor at least as high as said G protein coupled receptor binding peptide bind to said immobilized G protein coupled receptor;

10 (d) removing unbound members of said protein-peptide fusion protein display library;

 (e) incubating said bound protein-peptide fusion protein display library with antibodies which specifically recognize the protein portion of said protein-peptide fusion protein display library members under conditions such that said antibodies specifically bind to said protein-peptide fusion protein display library members;

15 (f) removing unbound antibodies; and

 (g) detecting said bound antibodies.

30. A method of claim 29, wherein said protein-peptide fusion protein display library is a maltose binding protein-peptide fusion protein display library and said antibodies are anti-maltose binding protein antibodies.

31. An enzyme-linked immunosorbant assay which comprises the steps of:

 (a) immobilizing a G protein coupled receptor onto a solid support;

5 (b) providing a protein-peptide fusion protein display library;

2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
309
310
311
312
313
314
315
316
317
318
319
319
320
321
322
323
324
325
326
327
328
329
329
330
331
332
333
334
335
336
337
338
339
339
340
341
342
343
344
345
346
347
348
349
349
350
351
352
353
354
355
356
357
358
359
359
360
361
362
363
364
365
366
367
368
369
369
370
371
372
373
374
375
376
377
378
379
379
380
381
382
383
384
385
386
387
388
389
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
409
410
411
412
413
414
415
416
417
418
419
419
420
421
422
423
424
425
426
427
428
429
429
430
431
432
433
434
435
436
437
438
439
439
440
441
442
443
444
445
446
447
448
449
449
450
451
452
453
454
455
456
457
458
459
459
460
461
462
463
464
465
466
467
468
469
469
470
471
472
473
474
475
476
477
478
479
479
480
481
482
483
484
485
486
487
488
489
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
509
510
511
512
513
514
515
516
517
518
519
519
520
521
522
523
524
525
526
527
528
529
529
530
531
532
533
534
535
536
537
538
539
539
540
541
542
543
544
545
546
547
548
549
549
550
551
552
553
554
555
556
557
558
559
559
560
561
562
563
564
565
566
567
568
569
569
570
571
572
573
574
575
576
577
578
579
579
580
581
582
583
584
585
586
587
588
589
589
590
591
592
593
594
595
596
597
598
599
599
600
601
602
603
604
605
606
607
608
609
609
610
611
612
613
614
615
616
617
618
619
619
620
621
622
623
624
625
626
627
628
629
629
630
631
632
633
634
635
636
637
638
639
639
640
641
642
643
644
645
646
647
648
649
649
650
651
652
653
654
655
656
657
658
659
659
660
661
662
663
664
665
666
667
668
669
669
670
671
672
673
674
675
676
677
678
679
679
680
681
682
683
684
685
686
687
688
689
689
690
691
692
693
694
695
696
697
698
699
699
700
701
702
703
704
705
706
707
708
709
709
710
711
712
713
714
715
716
717
718
719
719
720
721
722
723
724
725
726
727
728
729
729
730
731
732
733
734
735
736
737
738
739
739
740
741
742
743
744
745
746
747
748
749
749
750
751
752
753
754
755
756
757
758
759
759
760
761
762
763
764
765
766
767
768
769
769
770
771
772
773
774
775
776
777
778
779
779
780
781
782
783
784
785
786
787
788
789
789
790
791
792
793
794
795
796
797
798
799
799
800
801
802
803
804
805
806
807
808
809
809
810
811
812
813
814
815
816
817
818
819
819
820
821
822
823
824
825
826
827
828
829
829
830
831
832
833
834
835
836
837
838
839
839
840
841
842
843
844
845
846
847
848
849
849
850
851
852
853
854
855
856
857
858
859
859
860
861
862
863
864
865
866
867
868
869
869
870
871
872
873
874
875
876
877
878
879
879
880
881
882
883
884
885
886
887
888
889
889
890
891
892
893
894
895
896
897
898
899
899
900
901
902
903
904
905
906
907
908
909
909
910
911
912
913
914
915
916
917
918
919
919
920
921
922
923
924
925
926
927
928
929
929
930
931
932
933
934
935
936
937
938
939
939
940
941
942
943
944
945
946
947
948
949
949
950
951
952
953
954
955
956
957
958
959
959
960
961
962
963
964
965
966
967
968
969
969
970
971
972
973
974
975
976
977
978
979
979
980
981
982
983
984
985
986
987
988
989
989
990
991
992
993
994
995
996
997
998
999
1000

32. An enzyme-linked immunosorbant assay of claim 33, wherein said protein-peptide fusion protein display library is a maltose binding protein-peptide fusion protein display library and said antibodies are anti-maltose binding protein antibodies.

33. A method of claim 1, wherein said library of candidate compounds is a peptide library.

34. A method of claim 1, wherein said library of candidate compounds is a small molecule library.

35. A compound identified by a method according to claim 1.

36. A compound identified by a method according to claim 29.

37. A method of identifying a G protein coupled receptor signaling inhibiting peptide, which comprises:

(a) providing a peptide library based on a native G protein coupled receptor binding peptide;

5 (b) screening said peptide library for high affinity binding to said G protein coupled receptor; and

(c) selecting a member of said peptide library having binding to said G protein coupled receptor of higher affinity than that of the native peptide.

38. A method of claim 37, wherein said screening of step (b) is performed by testing for binding to an intact G protein coupled receptor.

39. A method of claim 37, wherein said screening of step (b) is performed by testing for binding to at least an intracellular fragment of a G protein coupled receptor.

40. A method of claim 37, wherein said G protein coupled receptor binding peptide of step (a) is a G protein subunit or fragment thereof.

41. A method of claim 40, wherein said G protein subunit fragment is from about 7 to about 70 amino acids long.

42. A method of claim 40, wherein said G protein subunit fragment is from about 7 to about 55 amino acids long.

43. A method of claim 40, wherein said G protein subunit fragment is from about 8 to about 50 amino acids long.

44. A method of claim 40, wherein said G protein subunit fragment is from about 9 to about 23 amino acids long.

45. A method of claim 40, wherein said G protein subunit fragment is about 11 amino acids long.

46. A method of claim 40, wherein said G protein subunit fragment is a G α subunit.

47. A method of claim 40, wherein said G protein coupled receptor binding peptide is a G α subunit carboxyl terminal peptide.

48. A method of claim 40, wherein said G protein subunit is a G $\beta\gamma$ dimer.

49. A method of claim 37, wherein said screening of step (b) comprises at least two sequential binding assays.

50. A method of claim 49, wherein at least one of said sequential binding assays is a competitive binding assay.

51. A method of claim 37, wherein said screening of step (b) is a competitive binding assay.

52. A method of claim 50, wherein said competitive binding assay is characterized by co-incubation of members of said peptide library with said G protein coupled receptor binding peptide.

53. A method of claim 51, wherein said competitive binding assay is characterized by co-incubation of members of said peptide library with said G protein coupled receptor binding peptide.

54. A method of claim 51, wherein said peptide library members are capable of providing a detectable signal.

55. A method of claim 37, wherein said screening is an enzyme-linked immunosorbant assay.

56. A method of claim 37, wherein binding to said G protein coupled receptor is determined by measuring a signal generated from interaction of an activating ligand with said G protein coupled receptor.

57. A method of claim 56, wherein activation of said G protein coupled receptor is determined.

58. A method of claim 56, wherein inhibition of said G protein coupled receptor is determined.

59. A method of claim 37, wherein said peptide library is a combinatorial peptide library.

60. A method of claim 59, wherein said combinatorial peptide library is a protein-peptide fusion protein library.

61. A method of claim 60, wherein said protein-peptide fusion protein library is a maltose binding protein-peptide fusion protein library.

62. A method of claim 37, wherein said peptide library is a peptide display library.

63. A method of identifying a G protein coupled receptor signaling inhibitor compound, which comprises:

(a) providing a library of candidate compounds to screen for binding to said G protein coupled receptor;

5 (b) providing a high affinity G protein coupled receptor binding peptide;

(c) screening said library of candidate compounds for high affinity binding to said G protein coupled receptor in competition with said high affinity G protein coupled

10 receptor binding peptide; and

(d) identifying a member of said library of candidate compounds having binding to said G protein coupled receptor of equal or higher affinity than that of the peptides of step (b).

64. A method of claim 63, wherein said screening of step(c) is performed by testing for binding to an intact G protein coupled receptor.

65. A method of claim 63, wherein said screening of step (c) is performed by testing for binding to at least an intracellular fragment of a G protein coupled receptor.

66. A method of claim 63, wherein said G protein coupled receptor binding peptide of step (b) is a G protein subunit or fragment thereof.

67. A method of claim 66, wherein said G protein subunit fragment is about 7 to about 70 amino acids long.

68. A method of claim 66, wherein said G protein subunit fragment is about 7 to about 55 amino acids long.

69. A method of claim 66, wherein said G protein subunit fragment is about 8 to about 50 amino acids long.

70. A method of claim 66, wherein said G protein subunit fragment is about 9 to about 23 amino acids long.

71. A method of claim 66, wherein said G protein subunit fragment is 11 amino acids long.

72. A method of claim 66, wherein said G protein subunit is a G α subunit.

73. A method of claim 66, wherein said G protein coupled receptor binding peptide is a G α subunit carboxyl terminal peptide.

74. A method of claim 66, wherein said G protein subunit is a G $\beta\gamma$ dimer.

75. A method of claim 66, wherein said screening of step (c) is an enzyme-linked immunosorbant assay.

76. A method of claim 63, wherein binding to said G protein coupled receptor is determined by measuring a signal generated from interaction of an activating ligand with said G protein coupled receptor.

77. A method of claim 76, wherein activation of said G protein coupled receptor is determined.

78. A method of claim 76, wherein inhibition of said G protein coupled receptor is determined.

79. A method of claim 63, wherein said library of candidate compounds of step (a) is a focussed library of candidate compounds based on the structure of the peptide of step (b).

80. A method of claim 63, wherein said library of candidate compounds of step (a) is a combinatorial library.

81. A method of claim 80, wherein said combinatorial library is a diverse small molecule library.

82. A method of claim 81, wherein said diverse small molecule combinational library comprises drug-like molecules.

83. A method of claim 81, wherein said diverse small molecule combinational library is a focussed small molecule library.

84. A method of claim 83, wherein said focussed small molecule library comprises drug-like molecules.

85. A method of claim 84, wherein the members of said focussed small molecule library are based on the chemical structure of the peptide of step (b).

86. A G protein coupled receptor signaling inhibiting peptide identified according to a method of claim 37.

87. A G protein coupled receptor signaling inhibiting compound identified according to a method of claim 63.

88. A method of inhibiting G protein coupled receptor signaling in a cell having a G protein coupled receptor which comprises administering to said cell a compound identified according to a method of claim 1.

89. A method of inhibiting G protein coupled receptor signaling in a cell having a G protein coupled receptor which comprises administering to said cell a compound identified according to a method of claim 37.

90. A method of inhibiting G protein coupled receptor signaling in a cell having a G protein coupled receptor which comprises administering to said cell a compound identified according to a method of claim 63.

91. A method of inhibiting G protein coupled receptor signaling which comprises contacting a compound with said G protein coupled receptor which interferes with binding of said G protein coupled receptor to its cognate G proteins.

92. A method for identifying a G protein coupled receptor signaling modifier compound, which comprises:

- (a) providing a peptide identified according to the method of claim 40, wherein said peptide is labeled to

5 provide a detectable peptide signal;

- (b) providing a library of candidate G protein coupled receptor signaling modifier compounds;
- (c) contacting said peptide with said G protein coupled receptor under conditions such that said peptide

10 binds to said G protein coupled receptor;

- (d) removing unbound peptide from said G protein coupled receptor;
- (e) measuring the signaling activity of said peptide-bound G protein coupled receptor and measuring said

15 detectable peptide signal;

- (f) contacting the members of said library of candidate G protein coupled receptor signaling modifier compounds with said peptide-bound G protein coupled receptor;

20 (g) measuring the signaling activity of said peptide bound G protein coupled receptor and measuring said detectable peptide signal;

- (h) determining whether said G protein coupled receptor signaling activity is increased or decreased after

25 contact with said candidate compound and whether G protein coupled receptor peptide binding is increased or decreased after contact with said candidate compound; and

- (i) identifying compounds for which contact with said peptide-bound G protein coupled receptor results in both an

30 increase in peptide binding to said G protein coupled receptor and an increase in G protein coupled receptor signaling and identifying compounds for which contact with

35 said peptide-bound G protein couple receptor results in both increase in peptide binding to said G protein coupled receptor and decrease a G protein coupled receptor signaling.

93. A method of claim 92, wherein the method for measuring said signaling activity of said peptide-bound G protein coupled receptor is selected from the group consisting of:

- 5 (a) measuring inositol phosphate accumulation;
- (b) measuring intracellular Ca^{2+} levels;
- (c) measuring transendothelial electrical resistance;
- (d) measuring stress fiber formation;
- (e) measuring ligand binding;
- 10 (f) measuring receptor expression;
- (g) measuring receptor desensitization;
- (h) measuring kinase activity;
- (i) measuring phosphatase activity;
- (j) measuring nuclear transcription factors;
- 15 (k) measuring all migration (chemotaxis);
- (l) measuring superoxide formation;
- (m) measuring nitric oxide formation;
- (n) measuring cell degranulation;
- (o) measuring GIRK activity;
- 20 (p) measuring actin polymerization;
- (q) measuring vasoconstriction;
- (r) measuring cell permeability;
- (s) measuring apoptosis;
- (t) measuring cell differentiation;
- 25 (u) measuring membrane association of a protein that translocates upon GPCR activation, such as protein kinase C;

30 (v) measuring cytosolic accumulation of a protein that
translocates upon GPCR activation, such as protein kinase C;
 (w) measuring cytosolic accumulation of a protein that
translocates upon GPCR activation, such as src; and
 (x) measuring nuclear association of a protein that
translocates upon GPCR activation, such as Ran.

94. A compound identified by the method of claim 1,
which comprises a peptide selected from the group consisting
35 of SEQ ID NOS:14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38,
40, 42, 46-105, 115-132 and 147-305.

95. A compound selected from the group consisting of
SEQ ID NOS:14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40,
42, 46-105, 115-132 and 147-305.

40 96. A minigene construct encoding a compound according
to claim 94.

97. A minigene construct encoding a compound according
to claim 95.

45 98. A method for providing a therapeutic G protein
coupled receptor signaling modifier peptide to a mammal
which comprises administering to said mammal an expression
construct which expresses a peptide according to SEQ ID
NOS:14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46-
105, 115-132 and 147-305.

50 99. A method for treating a disease state in which
excess G protein coupled receptor signaling is a causative
factor, which comprises administering a compound according
to claim 98.

100. A method of claim 98, wherein said peptide is
55 delivered by an expression construct.

101. A method of claim 100, wherein said compound is a
non-peptide drug.